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Key Points:
- Low and high molecular weight DOM fractions' decay fitted the reactivity continuum while humic substances presented nonmonotonic patterns
- Biodegradation patterns were consistent across sites, and the low molecular weight fraction drove the decay kinetics of bulk DOM
- Variability in DOM composition across sites was induced by summer drought and determined the biodegradation kinetics

Supporting Information:
- Supporting Information S1

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Biodegradation kinetics of dissolved organic matter chromatographic fractions in an intermittent river

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Abstract Controls on the degradation of dissolved organic matter (DOM) are complex but key to understand the role of freshwaters in the carbon cycle. Both the origin and previous degradation history have been suggested to determine DOM reactivity, but it is still a major challenge to understand the links between DOM composition and biodegradation kinetics. An appropriate context to study these links are intermittent rivers, as summer drought naturally diversifies DOM sources and sinks. Here we investigated the biodegradation kinetics of DOM in the main aquatic environments present in a temporary river. During dark incubations we traced the dynamics of bulk DOM and its main chromatographic fractions defined using LC-OCD: high molecular weight substances (HMWS), low molecular weight substances (LMWS), and humic substances and building blocks. Bulk DOM decay patterns were successfully fitted to the reactivity continuum (RC) biodegradation model. The RC parameters depicted running waters as the sites presenting a more reactive DOM, and temporary pools, enriched in leaf litter, as the ones with slowest DOM decay. The decay patterns of each DOM fraction were consistent throughout sites. LMWS and HMWS decayed in all cases and could be modeled using the RC model. Notably, the dynamics of LMWS controlled the bulk DOM kinetics. We discuss the mechanistic basis for the chromatographic fractions' kinetics during biodegradation and the implications that preconditioning and summer drought can have for DOM biodegradation in intermittent rivers.

1. Introduction

Organic matter degradation is a major component of the global carbon cycle [*Ciais et al*., 2013]. Analyzing degradation data with process-based biogeochemical models is essential to quantify the turnover of organic matter in terrestrial [*Manzoni et al*., 2012] and aquatic ecosystems [*Koehler et al*., 2012; *Arndt et al*., 2013]. Therefore, a number of models have been developed in order to describe the kinetics of organic matter decomposition in litter and soils [*Bosatta and Ågren*, 1995; *Koehler and Tranvik*, 2015], marine sediments [*Westrich and Berner*, 1984; *Middelburg*, 1989; *Boudreau and Ruddick*, 1991], and freshwater ecosystems [*Vähätalo et al*., 2010; *Koehler et al*., 2012]. Indeed, the decomposition of dissolved organic matter (DOM) in freshwaters has relevance at the global scale [*Cole et al*., 2007; *Tranvik et al*., 2009] and is known to be faster than in other Earth compartments [*Catalán et al*., 2016]. Hence, for a comprehensive understanding of the carbon cycling in freshwater ecosystems, it is fundamental to unravel the kinetics of DOM biodegradation.

Classical models of organic matter biodegradation include discrete models based on a constant decay rate for the bulk organic matter (first-exponential decay models [*Berner*, 1964]) and multicomponent models that separate organic matter into a few groups with a distinctive decay rate [*Westrich and Berner*, 1984; *Canfield*, 1994]. Alternatively, continuum models provide a more realistic framework that assumes a continuous distribution of an infinite number of reactive types [*Boudreau and Ruddick*, 1991; *Bosatta and Ågren*, 1995; *Koehler et al*., 2012]. However, although these models describe the behavior of DOM decay quite accurately, the lack of established links between biodegradation kinetics and the composition of DOM is a major shortcoming in our understanding of the carbon cycle in freshwaters [*Findlay and Sinsabaugh*, 2003]. Coupling compositional fractions with operational reactive types defined from the decay models is not straightforward.

DOM compositional fractions can be defined in terms of size or molecular weight [*Kothawala et al*., 2006], optical properties [*Fellman et al*., 2010], elemental composition [*Kellerman et al*., 2014], or functional groups [*Helms et al*., 2014], among others. Most of these techniques have been applied to assess the effects of

biodegradation on DOM composition, but usually the kinetics of compositional changes are not assessed [*Helms et al*., 2008; *Paul et al*., 2012]. Some studies using spectroscopic measurements have shown increases and decreases in DOM fluorescent fractions [*Guillemette and del Giorgio*, 2012; *Catalán et al*., 2013a; *Sleighter et al*., 2014], but assessing the contribution of different pools to bulk DOM decay remains essential to mechanistically describe the biodegradation process.

DOM compositional changes during biodegradation will be a function of the origin of DOM and the microbial community composition as well as of multiple physicochemical factors. These factors can influence both the composition and structure of the DOM (e.g., irradiation or pH) and also the biodegradation process itself (e.g., microbial function or temperature). However, the initial DOM composition has been shown to define DOM degradation patterns and subsequently the parametrization of the applied decay model. For example, significantly different parameters of the reactivity continuum model (RC) have been reported for DOM of clear and brown water lakes [*Koehler et al*., 2012], and for the DOM of algal and terrestrial sources [*Guillemette et al*., 2013]. Moreover, the character of the DOM sources is defined not only by its autochthonous or allochthonous origin but also by the previous processing they have undergone [*Catalán et al*., 2013a]. Therefore, the spectrum of systems along the inland water continuum should constitute a complex and heterogeneous mosaic of DOM reactivities.

Interestingly, such heterogeneities in DOM sources and processing can be relevant not only at a regional but also at catchment and even reach scales. For instance, a distinguishing feature of temporary rivers (i.e., waterways that recurrently cease to flow at some points along their course) is a high level of spatial habitat heterogeneity, which has its maximum during dry periods [*Gasith and Resh*, 1999; *Steward et al*., 2012]. In such periods, one may find a mixed sequence of running waters and isolated water pools over small distances, with each habitat presenting different sources of DOM and water residence times (and thus different degrees of degradation) [*Vazquez et al*., 2011; *von Schiller et al*., 2015]. In addition, since most temporary rivers are located in arid and semiarid regions, this heterogeneity is enhanced by a myriad of retention structures in response to high water supply demand [*Döll et al*., 2009; *Zarfl et al*., 2014]. Thus, during dry periods, temporary rivers turn into a very heterogeneous set of aquatic habitats, which may contain DOM with contrasting composition and degradation kinetics.

Here we aim to evaluate the biodegradation dynamics of DOM in the different aquatic environments present in a river undergoing summer drought (temporary pools, running water reaches and, a weir in its tail and dam). We analyzed the biodegradation patterns of bulk DOM and the compositional fractions in terms of size-exclusion chromatography by adjusting different decay models. We expect DOM decay kinetics to be different for each aquatic environment due to contrasting sources and degradation histories. Also, we aim to identify the chromatographic fraction driving the bulk DOM biodegradation patterns and to test if the reactivity continuum model (RC) is appropriate to capture the behavior of each independent fraction.

2. Materials and Methods

2.1. Study Site and Sampling

We collected water samples from different aquatic environments along the Fluvià river network (NE of the Iberian Peninsula) during September 2013. The Fluvià is a Mediterranean clear water river draining a predominantly calcareous watershed through mixed forests (79%), agricultural (18%), and urban (3%) areas. The Mediterranean climate has a dry and hot summer period during which the river flow gradually decreases, followed by flow cessation and the formation of isolated water pools. We selected different environments representative of the summer drought conditions linked to flow cessation, described to be diverse in terms of DOM sources and water residence time [*Casas-Ruiz et al*., 2016]. Along a 7 km reach of the main stem and upstream to downstream, we sampled two isolated pools of less than 25 m^2 (A_Pool and B_Pool; Table 1 and supporting information Figure S3), two running water reaches (C_Run and D_Run), and finally two points in a small weir, the first one in the river mixing point (E_WTail) and the second near the dam (F_WDam). Isolated pools were 500 m away from each other and were located in shaded areas under riparian canopy, both with visible accumulation of tree leaves. Discharge in running water reaches was 0.122 $m^3 s^{-1}$, with a water velocity of 0.07 m s^{-1}, and a width and mean water depth of 5.1 and 0.33 m, respectively. The area, volume, and water residence time of the weir were 1.24 hm^2, 0.0174 hm^3, and 1.65 days, respectively. The water depth in

Table 1. Location and Physicochemical Properties of the Sampling Sites[a]

Site	Location		DOC(mg C L^{-1})	TDN(mg N L^{-1})	T(°C)	pH	Cond(μS cm^{-1})	DO(%)
	UTM *x*	UTM *y*						
A_Pool	454408	4663864	3.9 ± 0.1	1.5 ± 0.1	16.4	7.3	344	17
B_Pool	454536	4664300	4.2 ± 0.1	1.5 ± 0.1	16.5	7.2	336	3
C_Run	454549	4665842	1.3 ± 0.0	3.6 ± 0.1	15.1	7.7	724	81
D_Run	454919	4666891	1.5 ± 0.2	4.9 ± 0.1	16.0	7.7	610	113
E_WTail	456045	4669233	2.4 ± 0.2	2.9 ± 0.1	16.9	8.2	522	97
F_WDam	456464	4669278	2.2 ± 0.3	4.1 ± 0.4	16.0	8.2	506	78

[a]DOC, dissolved organic carbon; TDN, total dissolved nitrogen; *T*, water temperature; Cond, conductivity; and DO, dissolved oxygen saturation.

the weir was 0.6 m in E_WTail and 3.9 m in F_WDam. Samples were stored in the dark at 4°C until the experiment started within 24 h of water collection.

2.2. Experimental Setup

Water was filtered through 1.2 μm precombusted filters to remove larger particles (Whatman GF/C) and then through 0.2 μm filters (nylon membrane filters, Whatman, UK). All filter units were rinsed with Milli-Q water before use, and care was taken to avoid high pressure during the filtration process to prevent bursting of cells and unintended release of DOM. Samples were incubated in 60 mL precombusted glass vials with an inoculum (1.2 μm filtered water of each corresponding site) added in a 1:10 *v/v* proportion. No differences were found for initial bacterial abundance between the different sites (see section 2.3 and supporting information Figure S2). Samples were replicated 3 times and incubated in the dark at 20°C for 65 days. Different subsets of vials were prepared and sacrificed at each experimental time (0 h, 6 h, 18 h, 2 days, 4 days, 7 days, 14 days, 28 days, and 65 days). The difference in dissolved organic carbon (DOC) between the initial and the final time was considered the biodegradable DOM (BDOM).

2.3. DOM Properties and Bacterial Abundance

Prior to qualitative and quantitative DOM analysis, incubated samples were refiltered through 0.2 μm prerinsed filters to eliminate any newly formed bacterial biomass. Dissolved organic carbon (DOC) concentrations were determined in a Shimadzu TOC-VCS (Shimadzu Corporation, Japan) by high-temperature catalytic oxidation. The detection limit of the analysis procedure was 0.05 mg C L^{-1}. All DOC samples were acidified to pH 3.5 with 2 *M* HCl and preserved at 4°C in the dark until analysis.

The DOM size fractions were characterized by liquid chromatography-organic carbon detection (LC-OCD) following the procedure described in *Huber et al*. [2011]. In brief, LC-OCD is an automated size-exclusion chromatography system (SEC), coupled to a combination of UV and IR organic carbon detection [*Huber et al*., 2011]. Filtered samples passed through a size-exclusion column packed with resin (Toyopearl HW 50S, volume of 250 × 20 mm). Phosphate buffer (0.029 *M*, pH 6.5) was used as eluent at a flow rate of 1.1 mL min^{-1}. DOC was detected by infrared absorbance of CO_2 after UV oxidation at 185 nm in a cylindrical UV thin-film reactor (Graentzel reactor). Fractions were identified by using fulvic and humic acid standards from the International Humic Substances Society [*Huber et al*., 2011]. Analysis of the fractions was done by using the software FIFFIKUS (DOC-Labor Huber, Karlsruhe, Germany). LC-OCD allowed to differentiate three DOM fractions as in *Graeber et al*. [2015]: (i) high molecular weight substances (HMWS) of hydrophilic character (e.g., biopolymers like polysaccharides, proteins, or amino sugars), (ii) humic-like substances and building blocks (HS and BB), acids containing carboxyl and phenolate groups, and their breakdown products with a similar retention time as humic and fulvic substances standards, and (iii) low molecular weight acids and neutrals such as alcohols, ketones, sugars, or amino acids, combined as the low molecular weight substances (LMWS) fraction (more details in the supporting information Figure S4).

Bacterial abundance was measured following the procedure detailed in *Porter and Feig* [1980]. In brief, bacterial cells were counted in 20 fields per filter with epifluorescence microscopy (Nikon Eclipse 80i, Japan) after appropriate dilution (5–200) and staining (15 min) of samples with DAPI (4,6-diamidino-2-phenylindole; 2 μg mL^{-1}) and subsequent fixation on black polycarbonate filters (0.2 μm and 5 μm; Nuclepore, Whatman).

2.4. Modeling DOM Biodegradation

DOM biodegradation kinetics can be described using multiple models. Here we applied a reactivity continuum model, which considers each compound as a reactive type assumed to degrade following a first-order kinetics. Therefore, based on the probability distribution of reactivities, different groups of compounds or fractions degrading at specific decay rates (k) can be defined too. The initial distribution of reactivities most frequently chosen is the Gamma distribution [*Boudreau and Ruddick*, 1991; *Arndt et al*., 2013], which captures very well the organic matter biodegradation dynamics observed in inland waters [*Koehler et al*., 2012].

Following an approach as in *Koehler et al*. [2012], we used a Gamma distribution and modeled the relative DOC concentration (DOC_t/DOC_0) over time (t) as

$$\frac{DOC_t}{DOC_0} = \left(\frac{\alpha}{\alpha + t}\right)^{\nu}$$

where the parameter α (h) controls the lifetime of the most reactive compounds [*Arndt et al*., 2013] or the apparent initial age of the bulk DOM [*Koehler et al*., 2012], and ν (unitless) determines the shape of the distribution and is related with the prevalence of recalcitrant compounds. Therefore, high ν and low α values indicate dominance of labile compounds in the DOM, while low ν and high α values indicate a dominance of refractory compounds and low degradability [*Arndt et al*., 2013]. Throughout the manuscript k (h^{-1}) states for the decay rate of DOM and from the fitted RC models, its decrease over the incubation time is calculated as $\nu/(\alpha + t)$ [*Koehler et al*., 2012]. Model parameters were estimated using nonlinear regression package *nlme* in R. In order to test the RC model assumption, we fitted the data also to simple models usually used in OC decomposition modeling—a linear regression and a single-pool exponential model [*Olson*, 1963]—and calculated the Akaike weights of the three models, following the procedure described in *Koehler and Tranvik* [2015].

To determine the model best describing the patterns of change of each fraction from the LC-OCD, we followed a similar procedure but including also generalized additive models (GAM) in order to identify nonmonotonic trends not considered in the exponential-based models [*Manzoni and Porporato*, 2007]. We interpret the GAM fit as a null model, a flexible function not affected by prior assumptions based on the exponential decay behavior. Therefore, this fit will not allow to obtain a decay rate describing the biodegradation of a DOM fraction but might identify consistent temporal trends across the incubations. GAM models were fitted using *mgcv* Package in R [*R Core Team*, 2015] with cubic regression spline as the smoothing function. The degree of freedom was automatically selected with generalized cross validation in the GAMs.

In order to assess the relationship of the LC-OCD fractions with the decay rates, we performed linear regression analysis. Moreover, to check that these relationships were independent of the effect of DOM concentration, we performed a semipartial correlation analysis. To do so, we calculated the residuals of a linear regression between the natural logarithm of k and DOC_t/DOC_0 and related them to the LC-OCD fractions contribution. Regression analyses were validated by examining residuals and colinearity between explanatory variables. Variable values in the text are given as mean $\pm$ standard error unless stated otherwise. All the analyses were performed using R 3.2.2 [*R Core Team*, 2015].

3. Results

3.1. Site DOM Characterization

Initial DOM concentrations and quality differed between sites (Table 1 and Figure 1). The temporary pools showed the highest DOC concentrations (4.21 ± 0.06 mg C L^{-1}), followed by the weir (2.51 ± 0.56 mg C L^{-1}) and the running water sites (1.34 ± 0.01 mg C L^{-1}).

The predominant chromatographic fractions in the initial samples were LMWS and HS and BB (Figure 1). The HMWS presented very low concentrations, being over the detection limit only in the pool sites (A_Pool: 0.31 ± 0.04 mg C L^{-1} and B_Pool: 0.32 ± 0.01 mg C L^{-1}). Also, A_Pool and B_Pool presented higher concentrations of the HS and BB fraction than of the LMWS fraction, while an opposite composition was found for running water sites, with higher values of LMWS than of HS and BB. In the weir sites, LMWS were at higher concentrations than HS and BB, but the concentration of HS and BB was much lower in the dam (F_WDam) than in the tail (E_WTail).

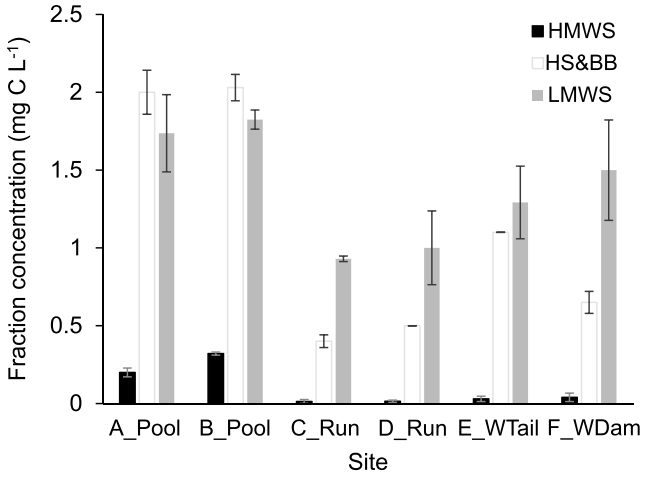

Figure 1. Initial composition of the DOM (mean ± SE) in the studied sites in terms of chromatographic fractions [*Huber et al.*, 2011]: high molecular weight substances (HMWS), low molecular weight substances (LMWS), and humic substances and building blocks (HS and BB). A_Pool and B_Pool refer to the two isolated pools, C_Run and D_Run to the running water reaches, E_WTail to the weir at its river mixing point, and F-WDam at its dam.

There was DOM consumption in all the incubations. The proportion of biodegraded DOM varied between 3 and 46%. The running waters showed the highest proportion of DOM biodegraded (42 ± 4% in C_Run and 41 ± 0% in D_Run), and lower values were found in the two pools (26 ± 1% in A_Pool and 32 ± 3% in B_Pool). In the weir, different amounts of DOM were consumed in the Tail (11 ± 8% E_WTail) and in the Dam (35 ± 5% F_WDam).

3.2. Bulk DOM Biodegradation Patterns

The different systems presented differentiated patterns in bulk DOM degradation, being similar for the two pools and the two running water reaches between them but not for the tail and dam of the weir (Figure 2).

The RC model properly described the degradation curves of the bulk DOM in all the sites (Figures 2 and 3 and Table 2). According to the model, the average lifetime of the most reactive compounds (α) was longer in the two pools than in the running waters or weir sites. Regarding parameter ν, related with the relative preponderance of the most recalcitrant compounds, the weir sites presented lower values than the pools and the running waters. All the sites showed high initial decay coefficients (Figure 3a and Table 2), but they were higher in the running water sites and the two points within the weir than in pools. k declined slowly over time in pools, while sites C_Run and E_WTail presented a very strong initial decrease in decay rates followed by B_Run and F_WDam. At the end of the incubation time, the apparent first-order decay coefficients of all the sites started to converge, reaching an average value of 0.0004 h^{-1} after 1 year. The cumulative distribution functions (Figure 3b) show the probability of a DOM molecule of having a specific initial reactivity. Accordingly, around 20% of the DOM in the running water sites is likely to decay at $k = 0.01\ h^{-1}$ or faster, while this fraction represents around the 13% in weir sites and only around 3% in the two pools (Table 2). The fraction degrading more slowly ($k < 0.001\ h^{-1}$) represents around 70–80% both in pool and weir sites, but only around 60% in running water sites.

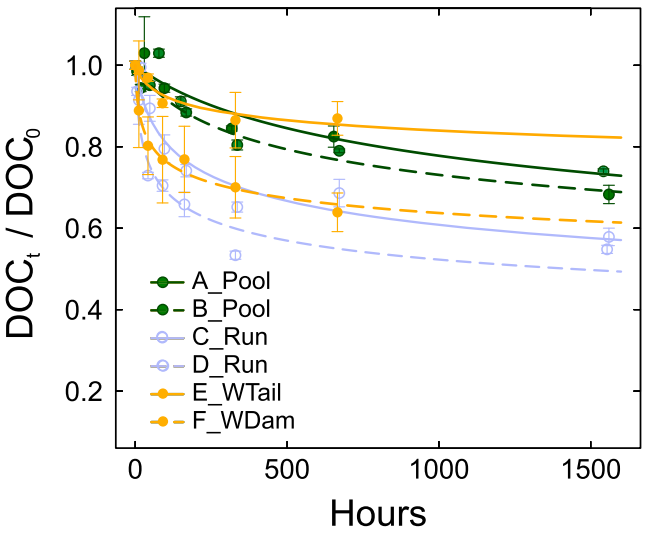

Figure 2. Time series of the relative decrease in dissolved organic carbon (DOC_t/DOC_0) during the incubations for the isolated pools A (A_Pool; green solid curve) and B (B_Pool; green dashed line), the running water reaches C_Run (blue solid curve) and D_Run (blue dashed line), and the weir at its tail (E_WTail; orange solid curve) and its dam (F_WDam; orange dashed curve). Mean and standard error values are shown. The lines show the values predicted by the reactivity continuum model.

3.3. Biodegradation Dynamics of the LC-OCD Fractions

The different fractions determined from LC-OCD presented very different patterns in time (Figures 4–6). LMWS and HMWS decreased, while the HS

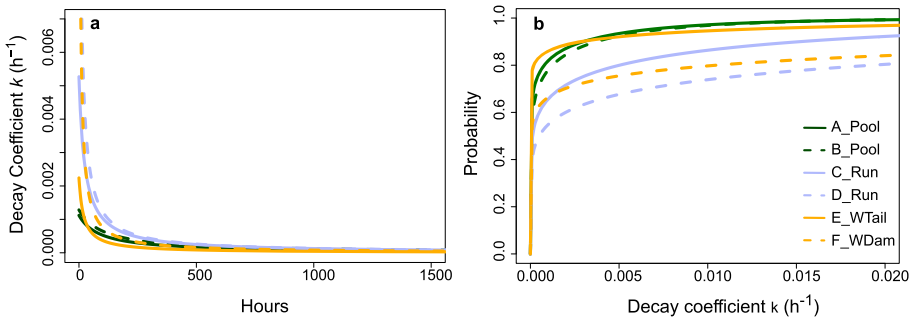

Figure 3. Output of the reactivity continuum model on bulk DOM. (a) Density functions of the models, showing the apparent first-order decay coefficient (*k*) over incubation time and (b) cumulative distribution function of initial reactivity for the isolated pools A_Pool (green solid curve) and B_Pool (green dashed line), the running water reached C_Run (blue solid curve) and D_Run (blue dashed line), and the weir at its tail (E_WTail; orange solid curve) and at its dam (F_WDam; orange dashed curve).

and BB fraction showed increases and decreases during the incubation time. These decreases reached up to the 60% of the initial LMWS (Figure 4) and up to the 80% of the HMWS (Figure 5). To gain insight into the fraction controlling the bulk DOM degradation pattern, we tested the relationship between the percentage of each fraction and the decay rates of the bulk DOM. Only the LMWS fraction showed a significant positive relationship with decay rates (Figure 7), and that relationship was independent of the bulk DOM concentration (Figure S1). We were unable to test this relationship for the HMWS, as this fraction was only available for two sites, and found no significant relationship with the HS and BB fraction.

The definition of the RC model as a continuous distribution of an infinite number of reactive types [*Boudreau and Ruddick*, 1991] implies that each compound conforming the DOM mixture decays following first-order kinetics. In agreement with that assumption, a reactivity continuum model could be fitted to the LMWS and HMWS fractions (supporting information Table S1). In both cases, in general, a better agreement was reached with the RC and GAM models than with the exponential or linear models (Figures 4 and 5; supporting information Tables S2 and S3) [*Wagenmakers and Farrell*, 2004]. The GAM model described slightly better than the RC decay of the LMWS fraction in sites A_Pool, D_Run, and E_WTail (Figure 4) and the HMWS fraction in site B_Pool (Figure 5). The parameters from the fitted RC models in these fractions are in accordance with the values obtained from the bulk DOM modeling (supporting information Table S1).

Regarding the HS and BB fraction, at the end of the incubation this fraction had decreased in A_Pool, B_Pool, and B_Run, while remained constant or increased in A_Run, E_WTail, and F_WDam (Figure 6). Throughout the incubation period, the HS and BB fraction showed increases and decreases and it was not possible to fit the gamma distribution of the RC model used. We further explored nonmonotonic trends using GAM models. We found significant trends (Figure 6); however, these trends were asynchronous and differentiated among sites, being impossible to find a common temporal pattern.

4. Discussion

4.1. Degradation Patterns of the Different DOM Fractions

The different DOM fractions presented consistent decay patterns across sites. Particularly, the LMWS fraction appeared to drive the biodegradation pattern of the bulk DOM in all the sites (Figure 7). LMWS are related to low molecular acids and low ion density molecules of amphiphilic or hydrophilic character, such as simple sugars or amino acids [*Huber et al*., 2011]. Therefore, this fraction should be readily available for uptake into bacterial cells. Indeed, it presents a high bioavailability (up to 60% of the initial DOM), as previously found in studies using SEC during biodegradation [*Paul et al*., 2012], and seems to be an important factor determining the decay rates of the bulk DOM (Figure 7). Moreover, the RC model, which assumes each compound decaying according to a simple exponential, was successfully fitted for LMWS in all the sites (Figure 4). The bacterial growth based on simple sugars and amino acids should translate in an exponential decrease of the concentration of each of these simple compounds [*Kaplan and Newbold*, 2003], in agreement with that model assumption.

Table 2. Estimated Parameters[a] of the Reactivity Continuum Models Fitted to the DOM Biodegradation Curves of the Different Sites

	A_Pool	B_Pool	C_Run	D_Run	E_WTail	F_WDam
α Average lifetime of the more reactive compounds (h)	89.5 ± 1.6	102.9 ± 1.4	24.1 ± 1.6	6.7 ± 1.95	19.8 ± 2.4	3.7 ± 0.6
ν Relative preponderance of the more recalcitrant compounds (unitless)	0.100 ± 0.012	0.132 ± 0.011	0.131 ± 0.012	0.133 ± 0.013	0.044 ± 0.014	0.080 ± 0.011
k Apparent initial first-order decay coefficient (h^{-1})	0.0011 ± 0.0007	0.0013 ± 0.0008	0.0054 ± 0.0007	0.0197 ± 0.0067	0.0224 ± 0.0057	0.0231 ± 0.0072
Reactivity class[b] 1 (%) $k > 0.01$	2.65	3.84	15.14	24.97	8.08	18.1
Reactivity class[b] 2 (%) $0.001 < k < 0.01$	15.37	18.66	20.41	15.61	10.85	16.17
Reactivity class[b] 3 (%) $k < 0.001$	81.99	77.5	64.45	59.42	81.07	65.72

[a]Model parameters reported with ± SE.
[b]Reactivity classes are defined as the proportion of initial DOM decaying with the defined k values [*Koehler et al*., 2012].

The HMWS fraction, previously related with fresh organic materials [*Graeber et al*., 2015], was also degraded to a large extent (up to 80%) and could be modeled with the RC model (Figure 5). The large molecular size of HMWS may indicate the presence of biopolymers, but it could also indicate the presence of supramolecular assemblies formed by several individual DOM compounds bonded through weak forces [*Piccolo et al*., 2002; *Peuravuori and Pihlaja*, 2004; *Schaumann*, 2006; *Kellerman*, 2015]. In both cases, these processes would fit well with the multiexponential decay underlying the RC. Biopolymer breakage, for example, is mediated through exoenzymes, leading to an exponential decrease of a particular substrate [*Sinsabaugh and Follstad Shah*, 2012]. High molecular weight compounds have often been related with humic colored and highly aromatic materials, of dubious biodegradation potential [*Stedmon et al*., 2007; *Helms et al*., 2008]. Comparison between size fractions obtained throughout different methods is complex due to methodological biases [*Nebbioso and Piccolo*, 2013], but in the case of LC-OCD, the hydrophilic character and absence of response of this fraction in the UV detector points toward biopolymers [*Huber et al*., 2011]. Therefore, the so called fast-decaying, short-term or labile fraction [*Guillemette and del Giorgio*, 2011; *Cory and Kaplan*, 2012] might in fact be the result of the biodegradation of both HMWS and LMWS representing, from aggregates to simple sugars, different stages in the disaggregation of biopolymers. This fast turnover related with biopolymers and monomers has been traditionally assessed during the study of the microbial loop in aquatic ecosystems [*Kirchman*, 2003; *Pomeroy et al*., 2007] and envisaged as being the fraction of carbon with the fastest turnover. Naturally, some compounds within these fractions are likely to present a more persistent character, especially in the case of LMWS. Small chromatographic fractions have been also associated with carboxylic-rich alicyclic molecules that tend to be preserved in natural waters [*Amon and Benner*, 1996; *Kellerman*, 2015].

Less well known are the kinetics of the compounds comprised in the last fraction, the HS and BB. The humic fraction (HS) comprises the humic acids containing carboxyl and phenolate groups produced by biodegradation of dead organic matter (MW of approximately 1000 Da). The BB, molecular chains of polyphenolics and polyaromatic acids that have disaggregated, likely breakdown products from HS [*Huber et al*., 2011]. It was impossible to fit this fraction to the RC, and it presented no clear temporal trends and a very poor fit to exponential or linear decays (Figure 6 and supporting information Table S4). Other studies on DOM biodegradation have reported simultaneous increases and decreases in different fluorophores, with increases mainly affecting humic-like regions of fluorescence, relating them with the simultaneous consumption and production of DOM during incubations [*Guillemette and del Giorgio*, 2012; *Catalán et al*., 2013a]. Moreover, in contrast with the bulk DOM kinetics and the LMWS and HMWS fractions, we did not clear temporal pattern on the HS and BB biodegradation across sites. These results point toward the uncoupling between the decay kinetics applied to the bulk DOM, mainly dominated by easily decomposable compounds that will decay according to the RC model, and the kinetics of humic substances that are likely to follow complex nonmonotonic trends as exemplified here with the fitting to GAM models (Figure 6). We do not aim to present GAM models as the analytical solution to the transformation processes that humic DOM suffers during biodegradation. The use of complex nonmonotonic models might lead to impractical multiple parameterizations hard to apply, thereby dampening the understanding of the OM decomposition process [*Manzoni et al*., 2012]. Our aim is instead to highlight the role of nonmonotonicity in the degradation of specific fractions of DOM, as has been shown for soil OM decomposition [*Neill and Gignoux*, 2006; *Manzoni*

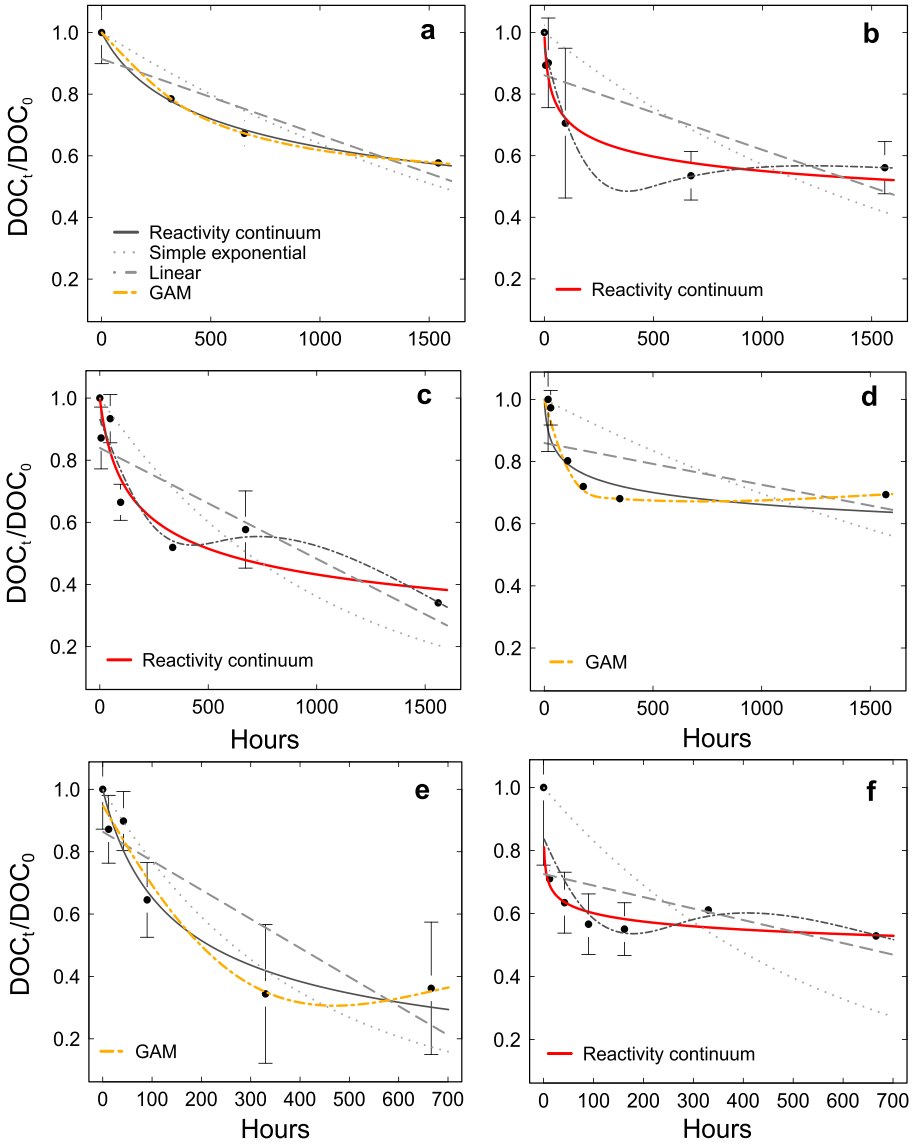

Figure 4. Relative decrease in the LMWS fraction (mean ± SE) during the incubations in the isolated pools (a) A_Pool and (b) B_Pool, the running water reaches (c) C_Run and (d) D_Run, and the weir (e) E_WTail and (f) F_WDam. The curves represent the predicted loss of LMWS modeled by the reactivity continuum model (continuous line), a simple exponential model (dotted line), a linear model (dashed line), and a GAM model (dash-dotted line). When colored and present in the legend, the line shows the model presenting the best fit according to Akaike weights (see supporting information Table S2).

and Porporato, 2007]. In that sense, the use of GAM models could prove very useful to identify temporal patterns along incubations. Mainly in short-term experiments, organic carbon can present a fluctuating behavior due to strong biotic-substrate interactions [*Manzoni and Porporato*, 2007] related with the activity of oxidative exoenzymes, which can be especially relevant in the degradation of humic substances [*Arnosti*, 2003]. For example, further research and an increased number of samples might help elucidate if the initial increase followed by a decrease in the HS and BB fraction (Figures 6b–6d and 6f) is consistent. To conclude, in the timescales of most incubation studies of freshwater DOM, a fluctuating behavior leading to nonmonotonic trends in the biodegradation of humic fractions might be expected.

4.2. Composition Effects on Bulk DOM Degradation Patterns

The sites presented differentiated patterns of DOM biodegradation that were related with differences in the initial composition and site particularities. These site particularities include factors such as DOM sources and

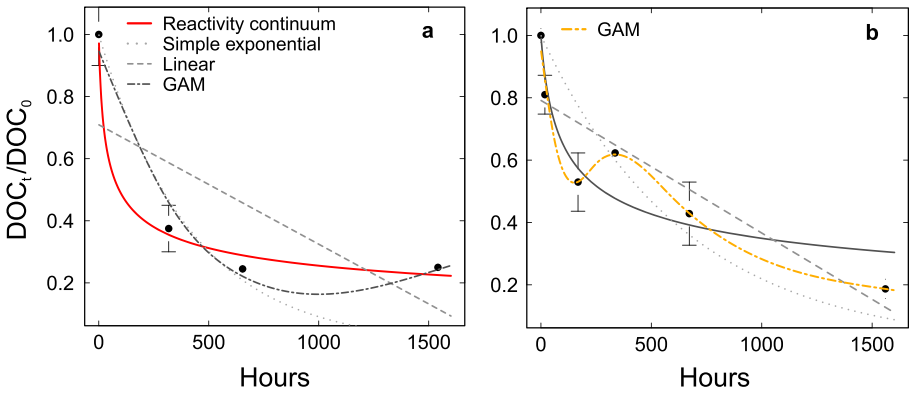

Figure 5. Relative decrease in the HMWS fraction (mean ± SE) during the incubations in the isolated pools (a) A_Pool and (b) B_Pool. HMWS concentration was under the detection limit in the other sites (Figure 1). The curves represent the predicted loss of HMWS modeled by the reactivity continuum model (continuous line), a simple exponential model (dotted line), a linear model (dashed line), and a GAM model (dash-dotted line). When colored and present in the legend, the line shows the model presenting the best fit according to Akaike weights (see supporting information Table S3).

history of degradation, microbial communities, water isolation, pH, temperature, or availability of inorganic nutrients which might define the structure and biodegradation potential of the DOM in natural conditions [*del Giorgio and Davis*, 2003]. Therefore, here we consider the differences in the biodegradation patterns among systems as the result of divergent DOM sources and also of their preconditioning across environments. In general, the biodegradation of DOC from the six sites was extensive (up to 40% BDOM in the two running water reaches) and in the fast range of incubation studies of river DOM elsewhere [*Del Giorgio and Pace*, 2008; *Fellman et al.*, 2014]. A common trait of DOM in Mediterranean systems is its low color and aromaticity [*Vazquez et al.*, 2011; *Catalán et al.*, 2013b; *von Schiller et al.*, 2015], which leads to comparatively higher biodegradation potential than in systems dominated by colored, strongly humic substances like the boreal systems [*Berggren et al.*, 2010].

DOM from the two upstream pools presented the slowest decay; the lifetime of the most reactive compounds was long and the values of the decay coefficients declined slowly, indicating a broad spectrum of reactivities for these systems [*Koehler and Tranvik*, 2015]. However, despite showing the slowest bulk DOM decay, the pools included a fraction of HMWS that presented high biodegradation (Figure 5). The HMWS fraction, also called biopolymers [*Huber et al.*, 2011], is related with high molecular weight compounds (>10 kDa [*Rutlidge et al.*, 2015]), usually polymers as polysaccharides with some contribution of nitrogen-containing materials such as proteins or amino sugars [*Huber et al.*, 2011]. Structural polysaccharides are major components of leaf litter detritus [*Mansfield and Baerlocher*, 2005; *Engelhaupt and Bianchi*, 2001], which accumulates in the studied temporary pools [*Casas-Ruiz et al.*, 2016]. Leaf litter leaching has been reported to increase the presence of large, highly biodegradable molecules [*Romaní et al.*, 2006; *Cuss and Guéguen*, 2015]. Temporary pools not only have strong leaf litter inputs but also a longer water residence time than the running waters and the weir, which leads to an increase of in situ microbial activity and autochthonous DOM sources [*Fellman et al.*, 2011; *Vazquez et al.*, 2011]. Therefore, these large compounds in the pools were likely linked with fresh litter leaching and concomitant microbial activity. Accordingly, this HMWS fraction has been linked with fresh organic materials previously [*Graeber et al.*, 2015]. However, pool samples also present an important fraction of HS and BB. This fraction represents different acids resulting from the biodegradation of dead organic matter [*Rutlidge et al.*, 2015] and may be related to the presence of a very large reactivity class with very low decay rates (Figure 3b and Table 2). Accordingly, as sugars from fresh litter leachate are consumed, the humic fraction might become relatively more abundant [*Cleveland et al.*, 2004]. The accumulation of the old running water DOM and the byproducts of leaf litter decomposition [*Casas-Ruiz et al.*, 2016] shapes pools as spots where OM undergoes a more extensive degradation. As a result, slow-degrading compounds such as HS and BB accumulate in temporary pools, to be flushed down once the rain reconnects them to the main flow.

The DOM from the running water reaches presented a marked decay with high initial apparent *k* and a low percentage of compounds with slow decay (Reactivity Class 3; Table 2). Running waters presented also the highest relative content in LMWS. This fraction includes both low molecular acids and neutrals which might

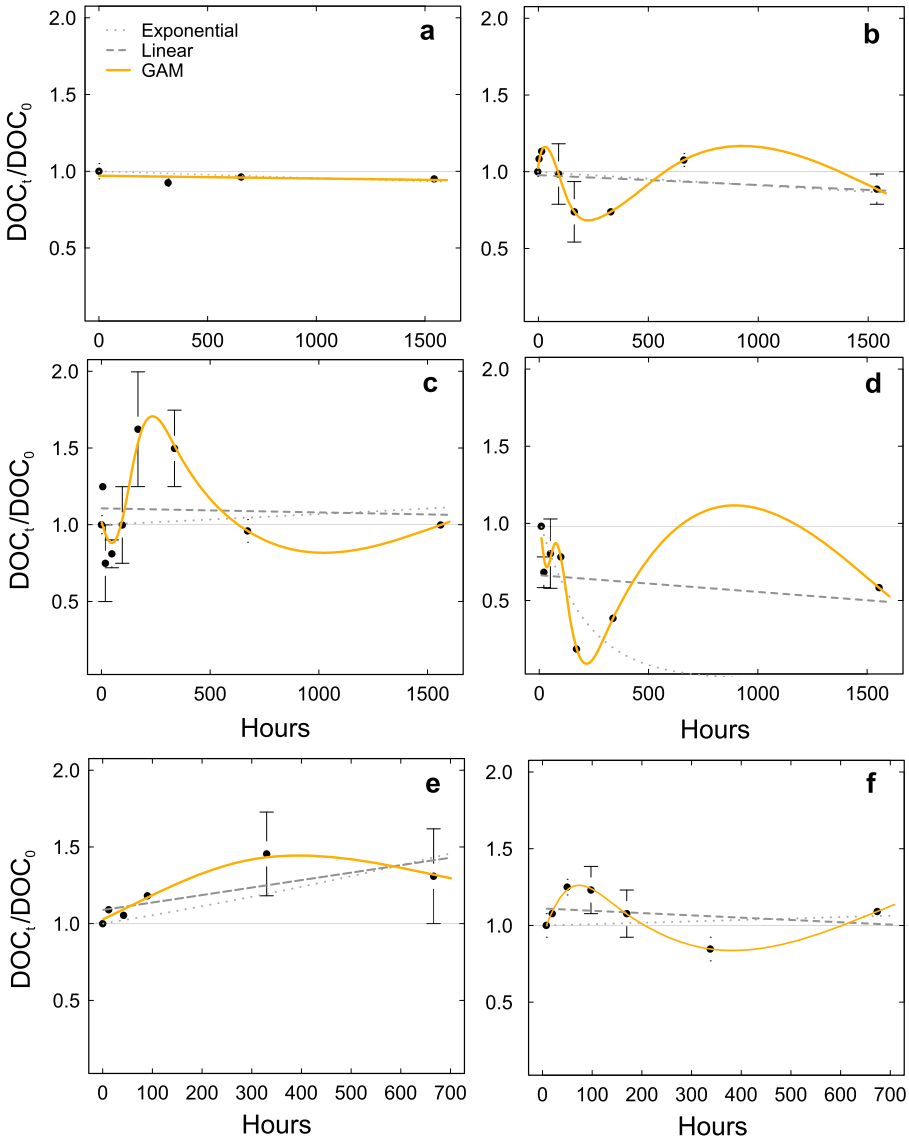

Figure 6. Relative decrease in the HS and BB fraction (mean ± SE) during the incubations in the isolated pools (a) A_Pool and (b) B_Pool, the running water reaches (c) C_Run and (d) D_Run, and the weir (e) E_WTail and (f) F_WDam. The curves represent the predicted loss of HS and BB modeled by a simple exponential model (dotted line), a linear model (dashed line), and a GAM model (dash-dotted line); the reactivity continuum model could not be fitted for this fraction. When colored and present in the legend, the lines show the model presenting a better fit according to Akaike weights (see supporting information Table S4). A grey continuous line at $y = 1$ (no change in DOC) is shown for clarity.

include aliphatic acids [*Kellerman*, 2015] and small humic and fulvic acids together with markedly biodegradable molecules like simple sugars or amino acids [*Her et al*., 2002; *Huber et al*., 2011]. Strong degradation of DOM from running waters has been reported previously [*Lapierre et al*., 2013] and might be enhanced by important background concentrations of nutrients (total dissolved nitrogen (TDN); Table 1). Indeed, despite the central role of the LMWS on the decay rates of bulk DOM (Figure 7), the variability around this relationship (Figure S1) points towards factors other than composition that might also be influencing the degradation, such as nutrients or the microbial community. The low residence time of DOM in running waters with the consequent low exposure to previous degradation pathways might enable the presence of LMWS. Therefore, running water reaches might be mainly transporting less processed material [*Raymond et al*., 2016]. Indeed, short water residence times correspond to a decrease in DOM processing, raising the instantaneous decay rates [*Catalán et al*., 2016].

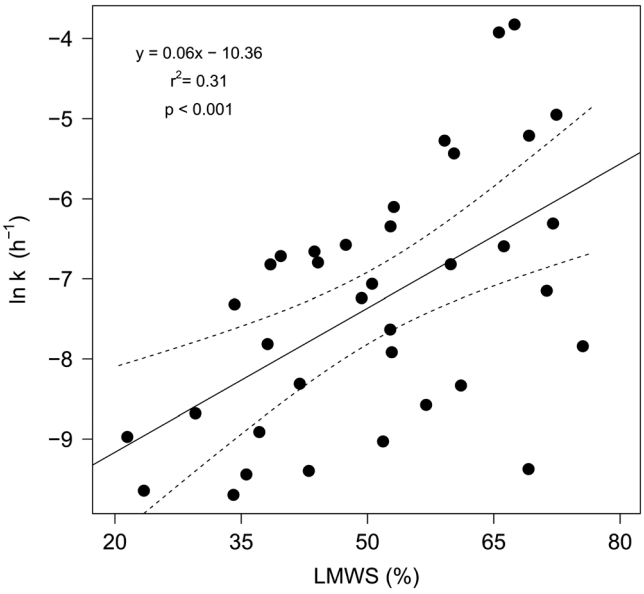

Figure 7. Relationship between the LMWS fraction and the modeled decay rates (*k*) at the corresponding incubation time in all the studied sites. Dotted lines show 95% confidence interval. An evaluation of the effect of DOC concentration on this relationship is presented in the supporting information Figure S1.

The two weir sites presented intermediate reactivity although differentiated degradation patterns. The weir in its tail (i.e., closer to the running waters) showed a degradation pattern similar to the pools (Figures 3a and 3b), whereas that of the site close to the dam was similar to the river sites. Taking into account the lower biodegradation (BDOM) and higher proportion of HS and BB in the E_WTail site, its reactivity pattern might be related to higher influence of the sediments. Indeed, the tail of weirs and impoundments, where river water mixes with the lentic water body, is strongly influenced by the activity of the accumulated sediment [*Cardoso et al*., 2013], especially during low flow periods [*Mosley*, 2015]. The F_WDam samples were taken close to the dam, where production in the water column is expected to increase. Therefore, sources of DOM will include in situ planktonic activity, and accordingly, increased biodegradation and LMWS were found. Small molecular weight compounds, such as aliphatic molecules with H:C ratio over 1 [*Kellerman*, 2015], have been related with aquatic production and found to increase with water residence time [*Köhler et al*., 2013], which would explain the divergence between E_WTail and F_WDam.

The divergences in composition and decay kinetics between the aquatic environments generated during drought, with enhanced degradation in temporary pools and increased primary production in weirs, would slow down the catchment-scale DOM decay rates. The severity of this intermittency is expected to increase in regions predicted to suffer decreases in runoff such as the Mediterranean [*Larned et al*., 2010; *Schewe et al*., 2014] and, in agreement with our observations, dry conditions have been predicted to generate a decrease in the OC decay rates [*Catalán et al*., 2016]. Intermittency is part of the idiosyncrasy of freshwater lotic ecosystems [*Steward et al*., 2012], and its role should therefore be included in the assessment of the DOM cycling at catchment scales.

5. Conclusions

The patterns found in bulk DOM decay were linked to compositional differences between the DOM isolated from the aquatic environments found in a river during summer drought. The lowest decay rates of bulk DOM were found in the temporary pools, systems strongly influenced by leaf litter inputs and likely to process DOM to a larger extent than river sites. In the case of weirs, the higher decay rates and higher proportions of LMWS might be linked to DOM sources derived from autochthonous primary production. The modeling of each chromatographic fraction showed a consistent pattern across sites, with LMWS and HMWS decaying rapidly and fitting to the RC model. LMWS showed a positive relationship with decay rates of bulk DOM suggesting that this fraction controls the overall kinetics of bulk DOM degradation. The HS and BB fraction did not present consistent degradation dynamics across sites, highlighting the need to further investigate the kinetics of compounds within that fraction to confirm nonmonotonic patterns. The divergences in DOM composition and degradation kinetics between the different aquatic environments show that intermittency affects the processing of bulk and compositional fractions of DOM in rivers.

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